DR. JANICE C FROEHLICH (Orcid ID : 0000-0003-2920-6937)

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Co-administration of low-dose naltrexone and bupropion reduces alcohol drinking in alcohol-preferring (P) rats

Emily R. Nicholson[1], B.S., Julian E. Dilley[1], B.S., Janice C. Froehlich[1], Ph.D.

[1]Indiana University School of Medicine, Indianapolis, IN 46202, USA

Correspondence: Janice C. Froehlich, PhD, Indiana University School of Medicine, 1120 West Michigan St., Room CL 380G, Indianapolis, Indiana 46202-5124; Tel.: 317-274-1347 Fax: 317-274-2695; Email jcfroehl@iupui.edu

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Background: This study examined whether combining naltrexone (NTX) with bupropion (BUP) is more effective in reducing alcohol drinking in alcohol-preferring (P) rats with a genetic predisposition toward high voluntary alcohol intake than is either drug alone.

Methods: Alcohol-experienced, adult, male, alcohol-preferring (P) rats were fed NTX alone in a dose of 10.0 mg/kg BW, BUP alone in a dose of 10.0 mg/kg BW, BUP alone in a dose of 20.0 mg/kg BW, NTX (10.0 mg/kg BW) + BUP (10.0 mg/kg BW), or vehicle (VEH) at 1 hour prior to onset of a daily 2-hour alcohol access period for 5 consecutive days.

Results: When administered alone, neither NTX (10.0 mg/kg BW) nor BUP, in either of two doses (10.0 mg/kg BW or 20.0 mg/kg BW), reduced voluntary alcohol intake in P rats. However, NTX combined with BUP (10.0 mg/kg NTX + 10.0 mg/kg BUP) and given as a single medication, significantly reduced alcohol consumption throughout prolonged treatment.

Conclusions: Combining low doses of NTX and BUP, each of which is ineffective when given alone, increases the efficacy of the medication. Low drug doses circumvent the problem of negative side effects that can occur with higher doses of either drug. A reduction in side effects can facilitate patient compliance and improve clinical outcomes for alcoholics and heavy drinkers who want to reduce their alcohol intake. The results, together with those from our prior studies, demonstrate the strength of a combinatorial pharmacotherapeutic approach to the treatment of alcohol use disorder (AUD).

Keywords: Alcohol Drinking, Alcohol Treatment, Selectively Bred Rats, Naltrexone, Bupropion

INTRODUCTION

One approach that has gained increasing popularity for the treatment of many disease states is combinatorial pharmacotherapeutics. When compared to monotherapies, the benefit of using combination therapies, involving more than one medication, is twofold. First, it allows drugs to be effective when administered in lower doses, which decreases the potential for

negative side effects that can limit patient compliance. Second, combining two drugs, each of which works via a different neurochemical mechanism, can target multiple processes and pathways that mediate the development and expression of a particular disease state. This approach is being adopted, with notable success, in the treatment of addictions such as alcohol use disorders (AUDs) (Farren et al., 2000; Froehlich et al., 2013b, 2016, 2017a; Heyser et al., 2003; Kiefer et al., 2003; Rasmussen et al., 2015; Ray et al., 2014).

Our research team has used a rodent model of alcoholism to test medications and identify those that can decrease alcohol drinking under a variety of conditions. Our approach has been to select FD- approved medications that have the potential to decrease alcohol drinking and alcohol self-administration when given in moderate to high doses, but that lack efficacy and aversive side effects at low doses. We have found that combining low doses of two such medications often restores efficacy while avoiding the potential for side effects. Given that naltrexone (NTX) is the drug most often used to treat AUD, we have combined low doses of NTX with other medications that differ from NTX in their mode of action (Froehlich et al., 2013b, 2016, 2017a; Rasmussen et al., 2015;). A similar approach is being used in humans (Kiefer et al., 2003; Ray et al., 2014).

The rodent model we employ was developed by Drs. Li and Lumeng who used a selective breeding approach to derive lines of rats that differ widely in the amount of alcohol they drink voluntarily (Li and Lumeng, 1979). The rat lines that drink large quantities of alcohol, referred to as the alcohol preferring or “P’ line and the high alcohol drinking or “HAD” line, are considered to be excellent animal models of alcoholism. Rats of the P line meet all of the suggested criteria for an animal model of alcoholism (Cicero, 1979). These selectively bred rat lines have been used to identify several drugs that can reduce alcohol intake in a variety of experimental paradigms (for review see Li et al., 1979, Froehlich and Li, 1991; O’Malley and Froehlich, 2003). In our early work, drugs were delivered via injection and the duration of drug treatment was necessarily short. In order to reduce the stress of drug administration,

and allow for prolonged drug treatment, we developed an oral drug delivery approach for rodents that is analogous to the way that drugs are delivered to humans (Froehlich et al., 2013a, 2013b, 2016, 2017a, 2017b; Rasmussen et al., 2015).

NTX is the most effective and extensively characterized medication currently available to treat AUD, and is considered the "gold standard" against which all other medications for AUD treatment are compared (for review see Froehlich et al., 2003; O'Malley and Froehlich, 2003). NTX is a nonselective opioid receptor antagonist that reduces the reinforcing effects of alcohol by antagonizing beta-endorphin-stimulated dopamine (DA) release in the nucleus accumbens (NAc) which is induced by alcohol (Di Chiara and Imperato, 1988; Imperato and Di Chiara, 1986; Koob et al., 1992). Subjects receiving NTX reported that the "high" they experienced from alcohol was less than they had previously experienced in the absence of NTX, and was less than they had expected to experience when they drank alcohol (Volpicelli et al., 1995). NTX decreases heavy drinking in both alcohol-dependent and nondependent drinkers as well as in young adults (for review see Chick et al., 2000; Hendershot et al., 2016; O'Malley et al., 2015) and reduces alcohol craving (Volpicelli et al., 1992). However, not all studies have found beneficial effects of NTX in the treatment of AUDs (Krystal et al., 2001).

Bupropion (BUP), a weak norepinephrine and dopamine reuptake inhibitor (NDRI), marketed as Wellbutrin® in United States and Canada, was originally approved by the FDA for treatment of major depressive disorder in 1985. Since then it has also been approved by the FDA for smoking cessation in 1997, for the prevention of seasonal affective disorder (SAD) in 2006, and for weight management in 2014. Currently, its role as an antidepressant remains its major medical application.

In contrast to many diseases, the appearance of which is restricted geographically, culturally or economically, AUD is widespread and ubiquitous. It is well recognized that individuals are compelled to drink alcohol for different reasons. Some drink to reduce anxiety, others to induce euphoria, still others to induce sedation and block memory of past events. Experienced alcoholics may also drink alcohol to alleviate withdrawal signs and symptoms. Successfully combating AUD requires an armamentarium of medications, which makes it essential that we continue to develop drugs that target the processes and pathways that underlie various motivations for alcohol drinking. The benefit of multiple pharmacotherapeutic treatment options is to increase the potential to reduce alcohol drinking in individuals who do not respond to a single drug alone.

We hypothesize that combining NTX with BUP will result in a medication that reduces alcohol drinking as effectively as have other drugs when combined with NTX such as prazosin (Froehlich et al., 2013b), varenicline (Froehlich et al., 2016, 2017a), and fluoxetine (Zink et al., 1997) and may help to fulfil the critical need for additional AUD treatment options.

MATERIALS AND METHODS

Subjects

Forty-two adult, male, alcohol-preferring P rats from the 77th generation of selective breeding for alcohol preference (P line) served as subjects. At the onset of the study, all rats were between 308 - 318 days of age. Lifespan is approximately 2 years in the rat and 79 years in the human. Rats that are 10-11 months old, as in the current study, are "comparable" to humans that are approximately 31 years old (Sengupta, 2013). The rats were individually housed in stainless steel hanging cages located in an isolated vivarium with controlled temperature (21+1°C) and lighting conditions (a 12 hour light/dark cycle with lights off at 0900 hours). Standard rodent chow (Laboratory Rodent Diet #7001, Harlan Teklad,

Madison, WI) and water were available ad libitum throughout the study. Fluids (water and the alcohol solution) were made available in calibrated glass Richter tubes. All rats had served as subjects in a prior study with NTX and varenicline that ended 3 months prior to the initiation of the current study. There is no evidence that residual effects of NTX or varenicline would be seen since the half-life of both drugs in the rat is quite short (1.8 ± 0.2 hours for NTX and 4.0 +/- 0.9 hrs for varenicline) (Obach et al., 2015; Hussain et al., 1987). All rats were maintained with food and water ad libitum and were given access to alcohol (15% v/v) for 2 hours a day for 3 months prior to initiation of the current study. All experimental procedures were approved by the Indiana University Institutional Animal Care and use Committee and conducted in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Alcohol Solution

A 15% (v/v) alcohol solution was prepared by diluting 95% alcohol (ethanol) with distilled and deionized water. The alcohol solution and water were presented in separate calibrated glass drinking tubes and daily fluid intakes were recorded to the nearest milliliter. Alcohol intake in milliliters was converted to g alcohol/kg body weight (BW) prior to data analysis.

BUP Palatability

We have previously fed NTX to rats in flavored gelatin stars and it is consumed rapidly and completely in a wide range of doses (Froehlich et al., 2013b, 2016, 2017a; Rasmussen et al., 2015). On the other hand, we have not previously fed BUP to rats. In a few prior studies, BUP has been administered orally by gavage (Hu et al., 2011; Welch et al. 1987) but it has not been delivered via voluntary consumption. Therefore, prior to feeding BUP to rats in the current study, an assessment of palatability was conducted in a separate group of 16 adult, male, alcohol-naive P rats (n=4/group) from the 78th generation of selection for alcohol

preference. All rats were fed one of four doses of BUP (0.0, 10.0, 20.0 or 30.0 mg/kg BW), in flavored gelatin stars, once a day for 5 consecutive days with food and water provided ad libitum. The percent of the gelatin star that was consumed within a minute was determined for each rat on treatment days and the average 5-day percent consumption is illustrated in Table 1. A Mann-Whitney Rank Sum test was used to compare intake in the vehicle vs drug-treated groups with significance accepted at $p<0.05$. Gelatin stars containing 0 (VEH) or 10.0 mg/kg BW BUP were entirely consumed. Consumption of stars containing 20 mg/kg BW BUP (96%, 96%, 92% and 100%, N=4) did not significantly differ from consumption of VEH stars (100%, N=4) but consumption of stars containing 30.0 mg/kg BW BUP was lower (95%, 95%, 82% and 87%, N=4; $p<0.05$) than was the consumption of VEH stars. In the current study, doses of BUP did not exceed 20.0 mg/kg BW in order to maintain palatability.

Drug Preparation

Naltrexone hydrochloride (NTX) and bupropion hydrochloride (BUP) (Sigma-Aldrich, St. Louis, MO) were dissolved in deionized and distilled water using sonication at 55^{o}C. The stock solution containing drug was added to a sweetened gelatin solution comprised of berry flavored Jell-O and gelatin in distilled and deionized water. NTX and BUP, expressed as free base masses, were added to the gelatin solution to provide the following doses: 10.0 mg of NTX/3.0 ml solution/kg BW, 10.0 mg or 20.0 mg BUP/3.0 ml solution/kg BW, and 10.0 mg NTX + 10.0 mg BUP/3.0 ml solution/kg BW. While still hot, the gelatin solution containing the drug(s) was aliquoted into star shaped molds with the volume of each aliquot determined by the final concentration of the gelatin (mg drug/ml of gelatin solution) and the BW of the animal in order to produce individual drug doses, 1 dose per day per rat, as previously described (Froehlich et al., 2013a, 2013b, 2016, 2017a, 2017b).

Oral Drug Delivery

The flavored, star-shaped pieces of gelatin (approximately 1.8 g each), containing NTX and BUP, alone or in combination, were fed to the rats once each day by inserting them through a hole in the front of the cage. The rats consistently ate the gelatin star within 1 minute. Cages were checked to confirm that no pieces of gelatin were dropped. On the rare occasion that rats dropped the gelatin star, the star was refed to the rat. The gelatin stars were fed each day at 1 hour prior to onset of the daily 2-hour alcohol access period due to the relatively short half-lives of NTX and BUP. The half-life of NTX in the rat is 1.8 ± 0.2 hours (Hussain et al., 1987). The half-life of BUP in the rat is 1.8 ± 0.1 hours (Al-Khamis et al., 1988). In the current study, gelatin stars without drug (VEH) were fed to all rats once a day for 3 days prior to the initiation of drug treatment in order to familiarize the rats with this oral drug delivery approach. We have previously used this approach for the prolonged daily administration of prazosin, NTX, and varenicline (Froehlich et al., 2013a, 2013b, 2016, 2017a, 2017b).

Assigning Rats to Groups

Rats were ranked in descending order of average daily alcohol intake for 2 consecutive days prior to the onset of drug treatment and were assigned to dose groups in a manner that ensured that alcohol intake did not differ between the groups at the initiation of drug treatment, as previously described (Froehlich et al., 2013a, 2013b, 2015, 2016, 2017a, 2017b).

Experimental Design

All rats were given access to alcohol (15% v/v) for 3 hours a day with food and water provided ad libitum. Rats were fed VEH, NTX (10.0 mg/kg BW), BUP (10.0 mg/kg BW), BUP (20.0 mg/kg BW), or NTX (10.0 mg/kg BW) + BUP (10.0 mg/kg BW) in gelatin stars at 1

hour prior to onset of the daily alcohol access period for 5 consecutive days. Alcohol intake and water intake were recorded after the first 2 hours of the daily 3-hour alcohol access period in order to facilitate comparison with our prior work. A daily 2-hour alcohol intake period has been used in all of our prior studies on the effect of oral NTX, alone and in combination with other drugs, on alcohol intake in P rats (Froehlich et al., 2013b, 2016, 2017a; Rasmussen et al, 2015a; 2015b).

Data Analysis

The effects of drug treatment on daily 2-hour alcohol intake during the 5 days of drug treatment were analyzed using two-way repeated measures analysis of variance (RM ANOVA) with repeated measures on day. The RM ANOVAs were followed by pairwise multiple comparisons using Dunnett's multiple comparisons against a single mean (VEH) to assess the effects of each drug dose vs VEH. When appropriate, Fisher's least squared difference (LSD) post hoc analyses were used to compare individual drug doses with each other. To assess how long the effect of drug treatment lasted after termination of treatment, a one-way ANOVA, one for each dose, was used to compare alcohol intake on post drug day 1.

The effects of drug treatment on water intake over the 5 days of drug treatment were analyzed in a manner similar to that used for alcohol, specifically, using a two-way RM ANOVA with repeated measures on day. The RM ANOVAs were followed by pairwise multiple comparisons using Dunnett's multiple comparisons against a single mean (VEH), to investigate significant effects.

Significance was accepted at $p<0.05$ and data are represented as means ± SEM. Consumption of less than three-quarters of a gelatin star resulted in the elimination of the drinking score for that rat on that day. Eliminated scores were replaced with the mean of alcohol intake for that rat on the day before and the day after the elimination occurred. Out

of a total of 630 scores in the study, only 6 eliminations were required: 5 in the 20 mg/kg BW BUP group (3 on day 1, 1 on day 2, and 1 on day 3), and 1 in the 10 mg/kg BW NTX + 10mg/kg BW BP (on day 1). Changes in body weight (g) over the course of 5 days of drug treatment were assessed for each rat (weight on day 5 minus weight on day 1/weight on day 1 X 100) and were compared using a one-way ANOVA.

RESULTS

Effect of NTX and BUP, alone and in combination, on 2-hour alcohol and water intake

With regard to alcohol intake, there was a significant effect of treatment [$F(4, 37) = 6.1$, $p<0.001$], day [$F(4, 148) = 13.4$, $p<0.001$], and no significant interaction (Fig. 1A). Dunnett's multiple comparisons against a single mean (VEH) revealed that only the combination treatment of 10.0 mg/kg BW NTX + 10.0 mg/kg BW BUP significantly reduced alcohol intake ($p<0.01$) (Fig. 1B). Fisher's LSD test, which compared all groups with each other, revealed that the only significant outcome was that the combination of NTX+BUP reduced alcohol intake when compared with VEH ($P<0.05$) with a trend toward reducing alcohol intake when compared with NTX alone ($p=0.058$) (Fig. 1B). Upon visual inspection of the data, there appeared to be a reduction of alcohol intake on days 1 and 2 of combined drug treatment (NTX+BUP) when compared to intake on days 1 and 2 of NTX alone. Therefore, separate one-way ANOVAs were performed on days 1 and 2 of drug treatment. On day one there was a significant effect of treatment [$F(4, 37) = 3.097$, $p<0.05$]. Fisher's LSD revealed that NTX+BUP reduced alcohol intake when compared to VEH, to 10 mg/kg BW BUP, and to 20 mg/kg BW BUP ($p<001$, $p<0.05$, and $p<0.05$, respectively) with a trend toward a reduction in alcohol intake when compared to NTX alone ($p=0.057$). On day 2 there was a significant effect of treatment [$F(4, 37) = 4.323$, $p<0.01$]. Fisher's LSD revealed that NTX+BUP reduced alcohol intake when compared to VEH, to 10 mg/kg BW BUP, and to 20 mg/kg BW BUP ($p<0.01$, $p<0.05$, and $p<0.001$, respectively) but not when compared to NTX alone

(p=0.143). With regard to alcohol intake after termination of drug treatment, there was no significant effect of treatment on postdrug day 1 (Fig. 1).

With regard to water intake during drug treatment, there was no significant effect of treatment, a significant effect of day [$F(4, 148) = 9.3$, $p<0.001$], and no interaction. Table 2 illustrates mean water intake in each treatment group during the 5 days of drug treatment.

Effect of NTX and BUP, alone and in combination, on body weight

There was no significant effect of treatment on body weight, when compared with VEH (Table 3).

DISCUSSION

To our knowledge, no prior studies have examined the effect of NTX+BUP on alcohol drinking in rodents and only a couple of studies have examined the effects of this combination on body weight. In the current study, when NTX and BUP were given alone in low doses, neither drug significantly reduced alcohol intake. However, combining NTX with BUP reduced alcohol intake independent of day. This decrease in alcohol intake was not accompanied by a change in body weight which was interesting in light of the fact that the combination of NTX and BUP, marketed as Contrave ®, was approved for weight management in 2014. Contrave ® is formulated as a single medication comprised of 8.0 mg NTX and 90.0 mg BUP. The absence of an effect of NTX + BUP on body weight in the current study agrees well with a prior report that NTX + BUP did not reduce body weight in rodents (Wright and Rodgers, 2013). The lack of an effect of NTX+BUP on body weight in the current study is likely due to the use of low doses of BUP and a relatively short treatment period.

Given the efficacy of BUP, marketed as Zyban®, in reducing smoking it is surprising that little research has been conducted to determine the effect of BUP on alcohol drinking since varenicline, marketed as Chantix®, which was developed to reduce smoking has also been demonstrated to reduce alcohol drinking in both animals and humans (Chatterjee et al., 2011; Froehlich et al., 2016, 2017a, 2017b; Lacroix et al., 2017; Litten et al., 2013 for review see Erwin and Slaton, 2014). This may be due to the fact that BUP is an antidepressant and many antidepressants have been shown to interact negatively with alcohol. For instance, tricyclic antidepressants, such as nortriptyline and clomipramine, have been reported to

exacerbate alcohol-induced drowsiness and impairment of motor function (Seppala et al., 1975) and selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine, have been reported to reduce the rate of alcohol abstinence in individuals with AUD (Kranzler et al., 1996; Le et al., 1999). Individuals taking these antidepressants are often advised to avoid alcohol. However, BUP does not exhibit the same side effects as the other classic antidepressants and does not augment alcohol-induced sedation or psychomotor impairment (DeVane et al., 1990). In fact, when BUP is taken with alcohol, reports of adverse neuropsychiatric events or reduced alcohol tolerance are very rare (Chandler and Herxheimer, 2011; Ramcharitar et al., 1992). Although, to our knowledge, there are no clinical studies directly examining the effects of BUP on alcohol drinking, it is important to note that BUP does not alter the euphoric effect of alcohol or alcohol "high" (Hamilton et al., 1984) and there does not appear to be a pharmacokinetic interaction between BUP and alcohol (Hamilton et al., 1984; Posner et al., 1984; Tartara et al., 1984).

The mechanism by which BUP may work to decrease alcohol intake is not clear but there is a general consensus that BUP increases extracellular concentrations of both dopamine (DA) and norepinephrine (NE) in the NAc by weakly blocking their respective transporters DAT and NET (Nomikos et al., 1989, 1992; Stahl et al., 2004). The level of DA in the NAc plays a role in mediating the reinforcing properties of alcohol that lead to alcohol drinking and increasing DA levels, through DAT inhibition by BUP, may account for a reduction of alcohol consumption (Di Chiara, 1999; Di Chiara et al., 2004; Thanos et al., 2001).

The fact that low doses of NTX + BUP reduced alcohol intake more effectively than either drug alone agrees well with our prior findings that low doses of NTX (10.0 mg/kg BW), that are not effective in decreasing alcohol intake when given alone, become effective when combined with other drugs such as fluoxetine (Zink et al., 1997), prazosin (Froehlich et al.,

2013b), or varenicline (Froehlich et al., 2016, 2017a). These drugs, which have different mechanisms of action, are all capable of potentiating the effect of low dose NTX.

The number of problems resulting from hazardous alcohol use continues to grow and alcohol use disorder (AUD) is among a few modifiable factors that contribute to early illness and death. Yet, according to recent analyses, less than a third of patients in AUD treatment programs, in both public and private sectors, are prescribed medication to treat AUD (Ducharme et al., 2006; Harris et al., 2013). This may be due, in part, to the limited number of FDA-approved drug treatment options currently available. Developing additional medications to treat AUD continues to be a top priority. Our laboratory has long maintained that combinatorial pharmacotherapeutics is a promising approach for reducing alcohol drinking in individuals who do not respond to a single medication alone. In our prior studies, using a rodent model of alcoholism, we have combined NTX, the "gold standard" for AUD treatment, with other drugs that have been FDA approved for treatment of anxiety and post-traumatic stress disorder (prazosin) (Froehlich et al., 2013a; 2013b), depression (fluoxetine) (Zink et al., 1997) and smoking (varenicline or Chantix®) (Froehlich et al., 2016, 2017a). We have found that these drug combinations can reduce alcohol intake when used in low doses that are not associated with aversive side effects.

Given that people differ in their motivation to drink (ie: to reduce anxiety, stress, or depression), drug combinations may be helpful for those who have found a single medication to be unsuccessful. The goal is to find ways to improve the outcome for individuals with hazardous drinking by identifying FDA-approved drugs that can be used in a combination that is tailored to address the particular need of subpopulations of alcoholics and heavy drinkers using a personalized medicine approach.

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FIGURE LEGENDS

Figure 1: (A) Effect of naltrexone (NTX), alone and in combination with bupropion (BUP), on alcohol intake on each of 5 days of drug treatment in P rats. **(B)** Effect of NTX, alone and in combination with BUP, on mean alcohol intake over the 5 days of drug treatment. Each point represents the mean ± SEM. ** $p<0.01$, vs VEH.

Table 1: Pilot Study - Percentage of Oral Drug Consumed over 5 Days of Drug treatment

Treatment	Percentage of oral drug consumed (%)
Vehicle	100%
10.0 mg/kg BW BUP	100%
20.0 mg/kg BW BUP	96%
30.0 mg/kg BW BUP	*87%

Table 2: Effect of Drug Treatment on Water Intake (ml/kg BW)

Treatment	Mean 2-hour water intake during 5 days of drug treatment
Vehicle	1.39 ± 0.28
BUP (10.0 mg/kg BW)	1.94 ± 0.43
BUP (20.0 mg/kg BW)	2.05 ± 0.32
NTX (10.0 mg/kg BW)	1.07 ± 0.28
NTX + BUP (10.0 mg/kg BW +10mg/kg BW)	1.15 ± 0.35

Table 3: Effect of Drug Treatment on Body Weight

Treatment	% Increase in body weight after 5 days of drug treatment
Vehicle	2.47 ± 0.2
BUP (10.0 mg/kg BW)	2.30 ± 0.3
BP (20.0 mg/kg BW)	1.73 ± 0.2
NTX (10.0 mg/kg BW)	2.05 ± 0.3
NTX + BUP (10.0 mg/kg BW +10mg/kg BW)	2.73 ± 0.4

TABLE LEGENDS

Table 1: Assessment of BUP palatability. Average percentage of bupropion (BUP) (0.0, 10.0, 20.0, 30.0 mg/kg body weight [BW]) consumed within one minute during the 5 days of drug availability. *p<0.05 vs VEH.

Table 2: The effect of naltrexone (NTX), bupropion (BUP), and NTX + BUP on water intake. Values represent the mean water intake (ml/kg BW ± SE) in each treatment group during the 5 days of drug treatment. Water intake was recorded daily 2 hours after the onset of the daily 2-hour alcohol access period.

Table 3: The effect of naltrexone (NTX), bupropion (BUP), and NTX + BUP on body weight (BW). Values represent percentage of increase in BW in each treatment group over the 5 days of drug treatment. BW was recorded once daily at the end of the daily 2-hour alcohol access period.